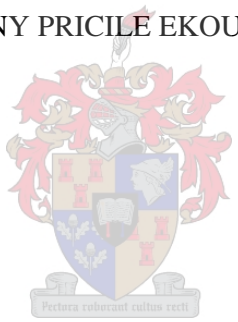
HOW EFFICIENT IS TECHNETIUM-99m LABELLING OF ERYTHROCYTES

IN PATIENTS WITH MALARIA

FANY PRICILE EKOUME



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DECLARATION

I, Fany Pricile Ekoume, hereby declare that the work contained in this assignment is my original work and that I have not previously submitted it, in its entirety or in part, at any university for a degree.

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ABSTRACT

With the expansion of Nuclear Medicine techniques in developing countries, it is essential to ensure a quality imaging procedure. In the case of red cell labelling, any factor which interferes with the labelling can lead to sub-optimal studies. With regard to the high incidence of malaria in sub-Saharan African countries in general and in Cameroon particularly, a high percentage of patients referred to Nuclear Medicine departments also have malaria. The question arose whether the presence of *Plasmodium* in erythrocytes or anti-malarial medication could affect the labelling of erythrocytes with technetium-99m.

The aim of this study was to investigate the impact of *Plasmodium* and anti-malarial medication on Tc-99m red cell labelling efficiency with in vitro kits in a population with a high prevalence of malaria infection.

Approval for this study was obtained from ethics committees of both institutions. Three groups of 30 patients were enrolled in the study after giving informed consent:

1. Smear-negative patients in an area where malaria is endemic (control group M-).
2. Patients with malaria as determined by a positive malaria smear test (group M+).
3. Patients with malaria and on anti- malaria medication (group Mm).

From each patient, a 5 ml blood sample was drawn in a heparinised blood collection tube. The red blood cells of each sample were labelled in vitro with Tc-99m, using an *in vitro* red blood cell kit. Labelling efficiency of the 3 groups was compared.

The average labelling efficiency was 98.2% ± 2.3% in malaria-free individuals, 98.6% ± 2.6% in patients with malaria but not on treatment, and 98.6% ± 1.1% in patients with proven malaria on quinine treatment. Non parametric data analysis using the Kruskal-Wallis ANOVA test for the percentage of labelling efficiencies showed a P-value of 0.2117 which was a confirmation that there was no significant difference between the labelling efficiencies for the three groups.

Radioactively labelled red blood cells are used in various nuclear medicine studies. Various drug therapies, including antibiotics, are known to either inflict direct or indirect damage to RBCs or their precursors or to impact influx or efflux of Tc-99m-pertechnetate into or out of RBCs, thereby decreasing labelling efficiency to such an extent that poor and inaccurate diagnostic information is obtained.

The results of this study indicate that malaria parasites and anti-malarial treatment with quinine do not affect in vitro erythrocyte labelling with Tc-99m, and should thus not interfere with nuclear medicine investigations.

OPSOMMING

Met die toenemende gebruik van Kerngeneeskunde in ontwikkelende lande is dit noodsaaklik om goeie kwaliteit beeldingsprosedures te verseker. In geval van rooiselmerking kan enige faktor wat met die merking inmeng tot sub-optimale studies lei. Weens die hoë insidensie van malaria in Afrikalande suid van die Sahara oor die algemeen en spesifiek in Kameroen, het 'n hoë persentasie van pasiënte wat na Kerngeneeskunde verwys word, malaria onder lede. Die vraag het ontstaan of die teenwoordigheid van *Plasmodium* in rooiselle of anti-malaria medikasie die merking van rooibloedselle met tegnesium-99m kan beïnvloed.

Die doel van hierdie studie was om ondersoek in te stel na die impak van *Plasmodium* en anti-malaria medikasie op die doeltreffendheid van rooiselmerking met Tc-99m met in vitro kitsstelle in 'n populasie met 'n hoë voorkoms van malaria infeksie.

Goedkeuring vir hierdie studie is van die etiese komitees van beide betrokke instansies verkry. Drie groepe van 30 pasiënte elk is by die studie betrek nadat hulle ingeligte toestemming gegee het:

1. Smeer-negatiewe pasiënte in 'n gebied waar malaria endemies is (kontrole groep M-).
2. Pasiënte met malaria soos bepaal deur 'n positiewe malaria smeertoets (groep M+).
3. Pasiënte met malaria en op anti-malaria medikasie (groep Mm).

'n Vyf ml bloedmonster in 'n gehepariniseerde bloedversamelingsbuis, is van elke pasiënt verkry. Die rooibloedselle van elke monster is met behulp van 'n *in vitro* rooiselkitsstel met Tc-99m gemerk. Merkingsdoeltreffendheid van die 3 groepe is vergelyk.

Die gemiddelde merkingsdoeltreffendheid was 98.2% ± 2.3% in malaria-vrye individue, 98.6% ± 2.6% in pasiënte met malaria maar sonder behandeling, en 98.6% ± 1.1% in pasiënte bewese malaria en op kinienbehandeling. Nie-parametriese data-analise met die Kruskal-Wallis ANOVA toets het 'n P-waarde van 0.2117 gelewer, wat bevestig het dat daar geen betekenisvolle verskil tussen die merkingsdoeltreffendhede van die drie groepe was nie.

Radioaktief gemerkte rooibloedselle word in verskeie Kerngeneeskunde studies gebruik. Dit is bekend dat verskeie middels, insluitende antibiotika of direkte of indirekte skade aan rooiselle of hul voorlopers veroorsaak, of die in- of uitvloei van Tc-99m in rooiselle beïnvloed, en sodoende die merkingsdoeltreffendheid in so 'n mate verlaag dat swak en onakkurate diagnostiese inligting verkry word.

Die resultate van hierdie studie toon dat malaria parasiete en anti-malaria behandeling met kinien nie die in vitro merking van rooibloedselle met Tc-99m beïnvloed nie, en dus nie met Kerngeneeskunde ondersoeke behoort in te meng nie

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My family and relatives.

Finally, my gratitude to **God**, the Almighty by whose grace my life is spared, my strength is full and sustenance is constant till this day.

DEDICATION

THIS BOOK IS DEDICATED TO GOD ALMIGHTY
AND MY PARENTS, SISTERS AND NEPHEW

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LIST OF ABBREVIATIONS

ACD-A:	Acid citrate dextrose solution formula-A
ACE:	Angiotensin-converting enzyme
ARbc:	Activity of red blood cells
As:	Activity of supernatant
ATP:	Adenosine triphosphate
CT:	Computer tomography
EDTA:	Ethylene diamine tetra-acetic acid
G:	gravity
HZ:	Haemozoin
HIV:	human immunodeficiency virus
KeV:	kilo electron volt
Kg:	kilogram
LE:	Labelling efficiency
K^+:	Potassium ion
M-:	Malaria free
M+:	With malaria
MBq:	Megabecquerel
Mm:	With malaria on quinine medication
ml:	millilitre
Na^+:	Sodium ion
NaOCl:	Sodium hypochlorite
RBC:	Red blood cell
RhD:	Rhesus D
Sn^{2+}:	Stannous ion
Sn^{4+}:	Stannic ion
$SnCl_2$:	Stannous chloride
SPECT:	Single photon emission computer tomography
Tc-99m:	Technetium-99m
TcO_4^-:	Technetium pertechnetate
TB:	Tuberculosis
YGH:	Yaounde General Hospital

CHAPTER ONE
GENERAL INTRODUCTION:

Labelling red blood cells with radionuclides is a common process recognized over the past 50 years [1]. The chemical structure of technetium allows it to be easily attached to a wide number of components including red blood cells. Preparation of Tc-99m labelled red blood cells is a well known technique in Nuclear Medicine. This preparation is commonly used for a number of investigations, in the diagnosis and follow up of a wide range of diseases [2, 3, 4, 5, 6]. It is especially useful in cardiovascular Nuclear Medicine.

Many reports indicate that Tc-99m labelling of red blood cells can be negatively affected by patient medication and other factors. This led to research on factors which could affect labelling efficiency. For example, Spicer et al incubated antineoplastic agents in vitro with whole blood and found that this did not interfere with the labelling efficiency of Tc-99m-RBC [7].

In recent years, sub-Saharan African countries have included more and more Nuclear Medicine departments in their healthcare policies with an increasing number of gamma cameras in hospitals. This includes increased use of Nuclear Medicine in tropical countries where malaria is endemic [8]. Thus there is an increasing possibility that patients referred to Nuclear Medicine also have malaria or could be on anti-malaria medication.

RBC infected by *Plasmodium falciparum* have been labelled in vitro by Tc-99m and used for experimental work in animals [9]. However this work did not discuss the labelling efficiency or the stability of Tc-99m-RBC binding. Currently no literature is available on the influence of malaria parasites or anti malarial medication on Tc-99m-RBC binding.

The current study addresses the question if the presence of *Plasmodium falciparum* in red blood cells and the action of the anti-malarial drug quinine on erythrocytes could affect labelling of red blood cells with technetium-99m.

CHAPTER TWO

LITERATURE REVIEW

MALARIA

Malaria is caused by *Plasmodium* parasites, spread to humans through the bites of infected *Anopheles* mosquitoes, which bite mainly between dusk and dawn. Four species are known to transmit the infection to humans, i.e. *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae,* and *Plasmodium falciparum*. Some human cases of malaria have also occurred with *Plasmodium knowlesi,* a South East Asian monkey malaria [8].

Following the infective bite, the incubation period in most cases varies from 7 to 30 days. The shorter periods are seen most frequently with *Plasmodium falciparum*. The classic malaria attack consists of a cold stage with sensation of cold and shivering, followed by a hot stage associated with fever, headaches, vomiting and seizures in young children, and finally a sweating stage with a to normal temperature. The severe form affects patient's blood and metabolism, and can include severe anaemia due to haemolysis, haemoglobinuria, and acute kidney failure [8].

Epidemiology:

The protozoa *Plasmodium falciparum* is the causative agent of the most virulent form of human malaria. More than two billion people are at risk for *Plasmodium falciparum* malaria worldwide [10], and more than 700,000 individuals die annually from this disease, predominantly in sub-Saharan Africa [11, 12].

Parasitology:

The overall biology of the life cycle of *Plasmodium falciparum* in both mosquitoes and mammalian hosts consists alternatively of invasive stages and of stages capable of intracellular asexual division. There is also a sexual stage of the life cycle which assists in the generation of genetic diversity within the parasite population.

Infection is initiated when sporozoites from the salivary glands of a female *Anopheles* mosquito are inoculated during a blood meal into the human bloodstream. These organisms invade hepatic parenchymal cells within an hour [13]. Once in the liver cells, the parasites initiate intracellular asexual multiplication. The products of the liver stage (the extra erythrocytic merozoites) are liberated from each parenchymal cell into the bloodstream. Here they attach to and invade circulating erythrocytes. Inside the erythrocyte, asexual division commences and over a period of 48 hours, the parasites develop through a series of

morphological changes from “ring” forms to trophozoites and finally to shizonts containing daughter erythrocytic merozoites. These are liberated by red cell lysis and immediately invade uninfected erythrocytes, producing a repetitive cycle of invasion and multiplication. The concomitant release of pyrogens is then responsible for the periodic “agues” which have long been a diagnostic feature of malaria infection and which occur at the time of merozoite release. The asexual blood forms are in fact the only forms of the parasite which give rise to clinical symptoms.

A minor proportion of merozoites within erythrocytes can undergo a different pathway of development in male and female gametocytes. Once fully mature, the gametocyte may return to the mosquito if ingested during a blood meal. Inside the mosquito midgut, male and female gametes are liberated from their host erythrocytes and fuse to form a zygote. This develops into an ookinete which is able to penetrate the gut wall and form an oocyst. At this point a further series of asexual divisions take place giving rise to sporozoites which migrate to insect salivary glands and thus complete the life cycle.

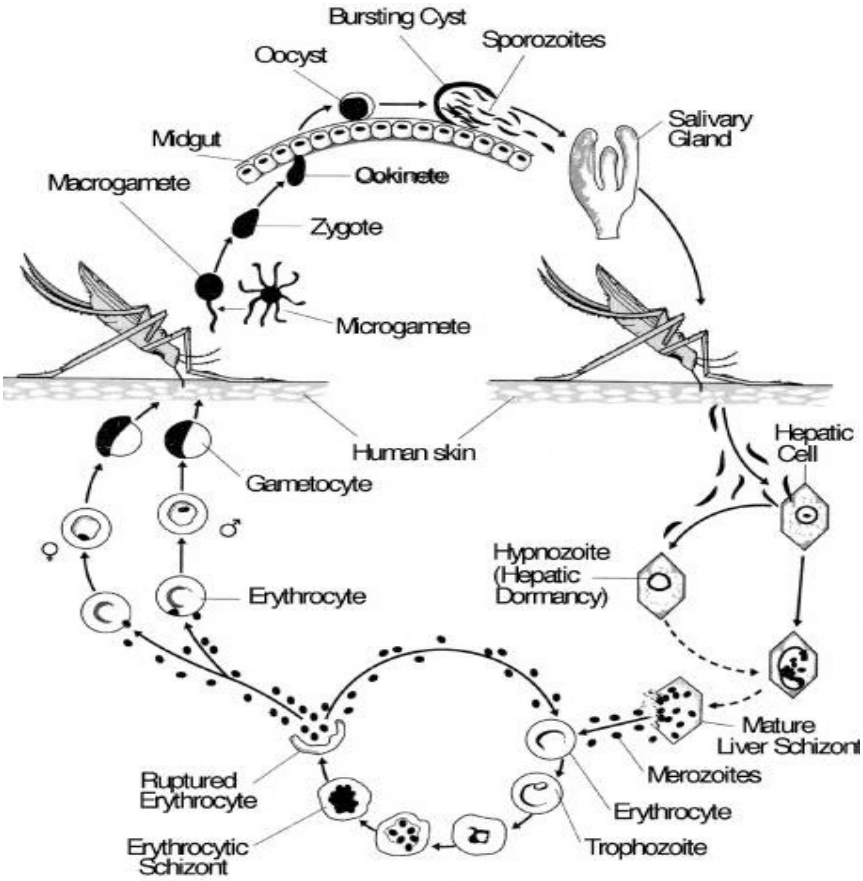


Figure 1: Life cycle of the malaria parasite in humans and the mosquito vector [13].

Biochemistry of *Plasmodium falciparum*:

The malarial parasite like all organisms acquires nutrients from the environment and converts these nutrients to other molecules and/or energy for its survival. For example the parasite requires amino acids for the synthesis of its proteins [14]. Haemoglobin is an extremely abundant protein in erythrocyte cytoplasm and serves as the major source of amino acids for the parasite.

Plasmodium falciparum export proteins into their host red cell cytoplasm [15].

During the digestion of haemoglobin, malaria parasites generate free haem upon catabolism of host haemoglobin in the erythrocyte. The haem moiety is not metabolized and accumulates in the parasite digestive vacuole. In order to minimize oxidative toxicity of the ferric iron, the free haem is polymerized into crystalline biomineral beta-haematin commonly referred to as haemozoin, also called malaria pigment. The haemozoin crystal becomes visible by microscopic examination during the trophozoite and schizont stages through the intra erythrocytic developmental cycle and continues to grow in size until the parasites are mature enough to egress the erythrocyte [14].

Pathogenesis:

It appears that the asexual intra erythrocytic stage of the life cycle of the parasite is responsible for the pathology of the disease. Erythrocyte invasion and effects of the parasite on the infected cell occur as follows:

The parasite (merozoite) is introduced in the erythrocyte via an initial attachment to the red blood cell, causing violent deformations and reorientation of the parasite. Once reorientation has occurred, invasion proceeds immediately. As a result of its entry and growth inside the red cell, a number of changes occur including:

Degradation of soluble host cell proteins mainly haemoglobin,

Changes in erythrocyte size and shape,

Alteration in the transport properties of the erythrocyte membrane,

Degradation of cytoskeletal components of the host cell,

Appearance of parasite specific antigens at the erythrocyte surface.

Sequestration is mediated by exposed parasite antigens at the cell surface [16].

Each merozoite after entering the red cell is transformed into a trophozoite which starts with successive asexual multiplications by nucleus division. In the end this causes the explosion of the cells with concomitant fever, destruction of the infected erythrocyte, liberation of

merozoite and invasion of new erythrocytes leading to successive endo erythrocytic cycles of *Plasmodium falciparum*. [13].

Pharmacokinetics and mechanism of action of quinine against *Plasmodium falciparum*:

Quinine is an important anti-malarial widely used for treatment of malaria caused by Plamodium Falciparum, especially in Sub Saharan Africa. [17, 18]. It is extracted from bark of *Cinchona officinalis*, *Cinchoma ledgeriana*, *Cinchona succirubra* or *Cincona calisaya* trees, of the family Rubiaceae. Common names include quinine bark, quina, quinine, kina kina, China bark, cinchona bark, yellow cinchona, red cinchona, Peruvian bark.

Quinine can be administered orally, but also parenterally. It acts as blood schizonticide and concentrated in food vacuole of *Plasmodium falciparum*. After oral administration it is 76 to 88% bioavailable with a half life of about 18 hours, metabolised in the liver and 20% excreted by urine. The antimalarial properties of the compound are thought to be a consequence of inhibiting parasite growth within infected erythrocytes.

Quinine like many other clinically used drugs is thought to act by inhibiting the formation of haemozoin in the food vacuole. Quinine inhibits nucleic acid synthesis, protein synthesis, and glycolysis in *Plasmodium falciparum* and can bind with haemozoin in parasitized erythrocytes. However, the precise mechanism of the antimalarial activity of quinine is not completely understood [19, 20, 21].

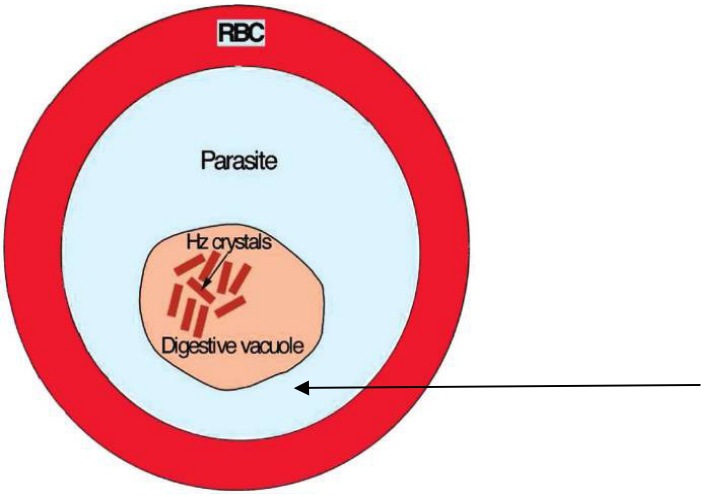


Figure 2: Schematic presentation of a RBC infected with malaria parasite, whose digestive vacuole is shown, containing mature haemozoin (HZ) crystals. (Diagram adapted from Weissbuch [13].

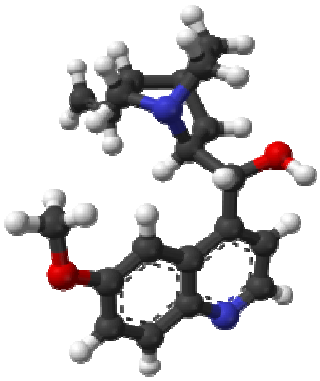


Figure 3: Quinine molecule [13]

RED BLOOD CELL LABELLING

Red blood cell anatomy:

In humans, mature red blood cells are flexible biconcave disks that lack a cell nucleus and most organelles leaving more space for haemoglobin. Haemoglobin is the largest pigmented protein responsible for gas transport [22]. Two point four million new erythrocytes are produced per second. They develop in bone marrow and circulate for about 100 to 120 days in the body before their components are recycled by macrophages, mainly in the spleen, bone marrow and liver. Their diameter is about 7.4 micrometers, the edge thickness 2.6 micrometers and the central thickness 0.8 micrometers [23].

Labelled red blood cells in Nuclear Medicine:

Fisher and his collaborators were the first to report radioactive labelling of erythrocytes with Tc-99m. Since their work several authors have conducted different investigations in the same field [24]. Steven M. Larson found that kit preparation of Tc-99m red blood cell provides an excellent blood pool imaging agent [25].

The availability of a Tc-99m procedure, using a commercial kit to label at high activity level a small volume of autologous blood permitted a suitable and non-invasive technique of measuring ventricular ejection fractions and regional abnormalities [26]. In addition, technetium emits gamma rays of 140 keV and has a physical half life of 6.02 hours; it has ideal properties that make it a radionuclide of choice for gamma camera imaging. The technique has even been used in newborn infants for red cell mass measurement [27].

An in vitro labelling method was described illustrating the predominant 20 hours blood clearance half time resulting in excellent image quality for over 24 hours which is an essential property for following intermittent gastrointestinal bleeding or for performing repeat cardiac function studies over a time interval of several hours [28]. It appears also to be a very good procedure for gated equilibrium blood pool imaging [29]. Bauer et al used in vivo / in vitro labelling of red blood cells with Tc-99m successfully in patients who had a radionuclide-ventriculography and those for localization of occult gastrointestinal bleeding and observed no adverse side effects [30].

Superiority of the in vitro labelling procedure over in vivo labelling for gated equilibrium blood pool imaging was demonstrated. Left ventricular function was continuously monitored using a radionuclide detector after intravenous injection of Tc-99m labelled red blood cells in patients with Parkinson's disease and postural hypotension. The author found that the study

may help to clarify the potential risk of sudden postural changes in such patients, which may cause fainting, syncope and increase risk of ischemic coronary and cerebrovascular attacks [31]. The feasibility of detailed evaluation of a flow characteristic in soft-tissue vascular malformations using Tc-99m labelled red blood cells was illustrated. Delayed liver SPECT/CT imaging after administration of Tc-99m red blood cells was found to be useful in the identification or exclusion of suspected hepatic haemangioma located near regions with high vascular activity [32].

There is an extensive list of authors who worked on the red blood cell labelling including findings on non-imaging Nuclear Medicine [33].

Red blood cell labelling principle and techniques:

During the erythrocyte labelling with Tc-99m, stannous ions (Sn^{2+}) diffuse into red blood cells and bind to the cellular components. This is called the "pre-tinning" of red blood cells. Pertechnetate (TcO_4^-) freely diffuses back and forth across the cell membrane of the erythrocytes. The Sn^{4+} ion and reduced forms of Tc-99m normally can not cross the cell membrane. Technetium reduced by Sn^{2+} can attach to the β- globulin chain of haemoglobin inside the erythrocyte [33, 34, 35]. This principle is applicable to in vitro, in vivo and in vivitro erythrocyte labelling procedures.

Different procedures of labelling:

In his comparative study of three labelling methods, Neumann and collaborators [36] concluded that in spite of the slightly higher technical investment involved in the in vitro labelling method this technique appears to be preferable for gated cardiac blood pool studies in view of the excellent labelling quality even though Creutzig in his letter to the editor indicated that the technique causes high radiation exposure to the staff [37].

a) in vivo red blood cell labelling:

The method consists of reconstitution of a vial of stannous pyrophosphate kit with sterile water for injection or sterile 0.9% normal saline solution. A concentration of 10-20 micrograms of stannous ion per kilogram of body weight is then injected to the patient [33]. A pre-tinning step of the red blood cells is thus done in the body by waiting 30 minutes after intravenous administration of the solution to the patient. Freshly eluted sodium pertechnetate

is then injected intravenously. Red cell labelling occurs within a few minutes in the circulating blood.

According to Early and Sodee [38], only about 75% of radioactivity is attached to red blood cells using the in vivo labelling process which is lower than what it is after in vitro labelling. The remainder will enter the extravascular space. This becomes a disadvantage in evaluation of gastrointestinal bleeding as sodium pertechnetate present in the blood will be secreted by the parietal cells of the stomach, causing false positive scans. But about 90% of the injected dose remained in the blood pool 2 hours after in vivo red cell labelling of rats and the findings suggested that red blood cells labelled in vivo with technetium may be as useful as those labelled in vitro for serial ejection-fraction studies and large vessel imaging [39].

b) In vivitro red blood cell labelling:

This is combination of both in vivo and in vitro methods where red blood cells are "pre-tinned" in vivo and labelled in vitro, with aim to remove unbound Tc-99m before injection. The radioactivity is measured in the method and thus the labelling efficiency can be calculated.

As described by Sampson [40], after a reconstitution of stannous agent with 0.9% sterile normal saline solution, 0.03ml/kg of stannous ion is intravenously injected. Fifteen to 30 minutes after the injection, 10 ml heparinised in vivo tinned blood sample is collected. Sterile normal saline 0.9% is then added to the blood sample followed by centrifugation at 500G during 5 minutes (washing step). The plasma is then removed and the washing step repeated to ensure removal of any extra-cellular stannous ions before adding the required amount of TcO_4^- to the cells. A 5 minute incubation time follows after which another washing step is done. The supernatant is removed and the labelling efficiency (%LE) is calculated as follows:

$$\%LE = \frac{ARbc}{ARbc + AS} x100$$

Where ARbc = Activity of red blood cells

AS = Activity of supernatant

Accurate results can be obtained with the in vivitro red blood cell labelling methods [40]. It could even produce labelling efficiencies as high as 95% [38].

c) In vitro red blood cell labelling:

The advantage of in vitro RBC labelling techniques is that extracellular tin can be removed before pertechnetate is added to the cells.

The clinical results of a new Tc-99m red blood cell labelling procedure avoiding cell centrifugation were performed. One ml of heparinized blood samples were incubated with kit containing stannous ion. By titration studies, ideal quantities of sodium hypochlorite for oxidation of extra cellular tin and of EDTA as stabilizer were used. Labelling efficiency remained high for two hours after injection. In addition the biological half life of labelled cells following the new procedure was 11 to 12 hours, rendering it suitable for serial determination radionuclide cardiography [41]. Superiority of the in vitro labelling procedure over in vivo labelling for gated equilibrium blood pool imaging was demonstrated using the Ultratag® RBC kit, which uses ACD-A and not EDTA [29].

This method usually requires the use of a lyophilized or frozen kit containing stannous ions. A red blood cell in vitro kit typically contains 50 µg stannous chloride dihydrate ($SnCl_2.2H_2O$), 3.7 mg sodium citrate, 5.5 mg dextrose and 2.0 mg sodium chloride.

One to two millilitres of heparinized blood are added to the kit. Five minutes incubation on a rotator mixer follows to let the stannous ions to enter the red blood cells and bind to the cellular components. After that sodium hypochlorite (NaOCl), an oxidizing agent, is added [42]. As NaOCl is unable to cross the red cell membrane, it will only oxidize the extra-cellular Sn^{2+} to Sn^{4+}. This is done to remove the excess tin in the extra-cellular medium. A citric acid/sodium citrate mixture (ACD-A) is added to sequester any residual extra-cellular stannous ions. The TcO_4^- recommended dose to add to the kit is 370-3700 MBq with an incubation time of 20 minutes. The activity of TcO_4^- to add to the red blood cells will depend on the clinical application. As described in the package insert of the commercially available Ultratag® kit, Tc-99m labelled red blood cells should be injected to the patient at this stage [43]. With the method described by Le Roux [44] and Lackay [45] which is the same method used at Tygerberg Hospital, the vial is centrifuged after labelling and plasma removed from the packed red blood cells. Any unbound Tc-99m will be removed with the plasma. The labelling efficiency is determined after measurement of the activity of the supernatant as well as the activity in the red cell pellet. The percentage of Tc-99m incorporated by the red blood cells, which is also called labelling efficiency (%LE), is calculated using the formula described above for in vivitro labelling. The red cell pellet is resuspended with sterile normal saline (0.9%) solution prior to injection.

The indication for the referral of the patient will help to decide about the methods of choice. In vivo red cell labelling is convenient as it avoids the direct handling of blood which can be a source of infectious contaminations. It consumes less time than the in vitro method. Unfortunately the in vivo method generally renders lower labelling efficiencies, which limits the usefulness of the method for many applications [36]. For example it is imperative to ensure a high labelling efficiency with quantitative applications such as blood volume studies. Furthermore in the detection of haemoptysis, poor labelling efficiency will lead to uptake of unbound radioactivity in the stomach, which in turn could result in false positive studies. The in vitro labelling method would be the method of choice for the above case.

Drugs and disease states affecting red blood cell labelling efficiency:

There is considerable evidence that the biodistribution or kinetics of radiopharmaceuticals may be altered during a disease state as well as due to drug consumption, even when they are used as vegetable extracts [46, 47, 48, 49 50]. Radiolabelling red blood cells with Tc-99m can be altered by interaction of synthetic and natural drugs [51, 52, 53, 54, 55, 56, 57], in some cases resulting in poor diagnostic information [58].

Drug interaction as cause of sub-optimal percentage of labelling was suspected with nifedipine in combination with other drugs [59], and medication such as doxorubicin cause abnormal cardiac distribution [60]. The same decreased effect was found with prazosin as well as digoxin [61].

To summarise poor labelling efficiency's causes, Sampson [1] presented a list including:

- Heparin anti-coagulant rather than acid-citrate dextrose solution A.
- Oxidation of Tc-99m with exposure to air
- Inadequate administration of stannous ion
- Prior administration of intravenous contrast media
- Patients with sickle cell anaemia
- Incubation with anti-RhD serum
- Anti-hypertensive therapy
- Formation of red blood cell antibodies
- Interference of drugs such as nifedipine, verapamil.

Gleue et al [62] incriminated antibiotics (gentamycin) and anti-inflammatory agents.

More detailed studies attempting to clarify the mechanism of interaction with red cell labelling were done by a Brazilian group of researchers. Morphological changes of

erythrocytes including membrane alteration did not always imply or correlate interference on the labelling of blood constituents with Tc-99m. Moreno and co-workers [63] incubated blood from Wistar rats in vitro with *Nectandra membranacea* extract at different concentrations. They added successively $SnCl_2$ and sodium pertechnetate. A morphometry study was simultaneously conducted by using a drop of each sample for smear tests on glass slides for microscopic evaluation with comparison to control blood (blood plus 0.9% saline, $SnCl_2$ and sodium pertechnetate). Qualitative analysis of the shape of RBC in samples incubated with *Nectandra membranacea* has shown important qualitative morphological alteration mainly in samples treated with highest concentrations, with P-value smaller than 0.05. The radioactivity in each sample was determined in a sodium iodide well counter and the percentage of administered radioactivity was calculated. The result showed no significant difference of values of treated samples with different concentrations of extract and control samples ($P>0.05$).

Similar results were found from work with *Sechium edule* [64] where in addition to morphological and radiolabelling investigation, electrophoretic mobility of plasmid from Wistar rats was studied. In spite of demonstration of microscopic morphological alterations and proof of damaging plasmid DNA by electrophoresis, *Sechium edule* extract was not capable of modifying the pattern of radiolabelling of blood elements with Tc-99m. The authors suggested that these extracts have anti oxidant compounds which could probably be responsible of alteration of the morphology of red blood cells without altering the radiolabelling of blood elements [65].

Another animal model study on female Sprague Dawley rats documented the effect of *Bacopa monnieri* (L) extract on the labelling of blood elements with Tc-99m and on morphology of red blood cells [66]. In this case, there was alteration of the morphology with a significant decrease of percentage of blood cell labelling. The authors suggested that the effect of *Bacopa monnieri* (L) could be explained by an inhibition of stannous and pertechnetate ions or oxidation of the stannous ion or by damages induced in the plasma membrane.

A report of an in vitro animal model study on influence of biflorin, a potent o-quinone antimicrobial product, was documented [67]. From four Wistar rat blood samples incubated at different concentrations of biflorin with $SnCl_2$ and sodium pertechnetate, the distribution of radioactivity in soluble and in soluble fractions of RBC was recorded. The result indicate an extreme decrease ($P<0.001$) in radioactivity fixation in the insoluble fraction of red blood cells only when 0.25mg of bioflorin is added to 0.5ml of blood sample as well as when

0.125mg of bioflorin in 0.5ml of blood. However, this work done in animal models differs from the situation in patients regarding the volume of blood used, and the actual radiolabelling method. The number of animals used per experiment is also very small. It is not clear how these experiments would translate into the clinical situation in diagnostic Nuclear Medicine.

A more optimal study in humans was done by Lackay [45] where the labelling of erythrocytes with Tc-99m was evaluated in patients who were treated with a cocktail of anti-tuberculosis drugs. Medicines used in the study included a combination of rifampicin, isoniazid pyrazinamide and ethambutol in the first phase of tuberculosis treatment, followed by a four months continuation phase of treatment with rifampicin and isoniazid only. Labelling efficiency in each treatment phase was compared with each patient's baseline RBC labelling before treatment. No statistically significant difference was found between the baseline studies and the labelling done during the two phases of treatment.

Thus from a review of the literature regarding the influence of medication and plant extracts on red blood cell labelling the following emerges:

1. From case studies and small series of observations in patients, it appears that many drugs can interfere with RBC labelling with Tc-99m.
2. Experimental work has been done on the effect of several plant extracts in rats, but it is not clear how this would relate to the situation of patients in nuclear medicine.
3. Very few detailed studies have been done in humans.

With a very high prevalence of malaria in Cameroon and in other Sub-Saharan African countries and the fact that the area is endemic for the disease [11, 12, 18], there is potential that patients referred for Nuclear Medicine investigations with radiolabelled red blood cells, may be infected with malaria and/or on anti malarial therapy. In view of a rich documentation on adverse effects due to both disease states and drug interactions on red blood cell labelling, the question arises if malaria or quinine would affect the labelling efficiency. However no published work could be found with respect to the possible influence of *Plasmodium falciparum* and antimalarial drugs on red cell labelling with technetium-99m.

Problem statement

With the expansion of Nuclear Medicine facilities in developing countries, it is essential to secure a high quality imaging procedure. This includes ensuring that any radiopharmaceuticals prepared for the patients are safe and effective. In the case of red cell labelling, any factor which interferes with the labelling can lead to sub-optimal studies. With regard to the high incidence of the malaria in sub-Saharan African countries in general and in Cameroon particularly, a high percentage of patients referred to Nuclear Medicine departments also have malaria. The question to be answered is whether the presence of *Plasmodium* in erythrocytes or anti-malarial medication could affect the labelling of erythrocytes.

AIM OF THE INVESTIGATION

The aim of this study is to:

- determine whether the presence of *Plasmodium falciparum* in red blood cells and
- the action of the anti-malarial drug quinine on erythrocytes

could affect labelling of red blood cells with technetium-99m.

CHAPTER THREE
MATERIALS AND METHODS

This study was approved by the Stellenbosch University Health Research Ethics Committee (No: N11/01/022) and Cameroon National Ethics Committee (No: 163/CNE/SE/2010) as well as the authorisation of the General Manager of Yaoundé General Hospital.
The participants gave a written informed consent before being enrolled into the study. The patient information sheet was available in English and French.

Study population:
A prospective study was conducted at the Yaoundé General Hospital (YGH) Nuclear Medicine Department, Haematology unit and blood bank unit, and hospitalisation units in Cameroon.
The blood samples used were collected with convenience sampling from day to day patients in consultation at YGH and at the "clinique mere et enfant" in Yaoundé, and from hospitalised patients in both centres.

Inclusion criteria:

- Consenting males and females aged between 18 and 60 years
- Patients free from HIV
- Patients free from sickle cell disease
- No chemotherapy and/ or radiotherapy during the last 6 months

Exclusion criteria:

- On request of the patient
- Patients on drugs known to interact with red blood cell labelling
- Patients with psychiatric illness
- Patients with chronic diseases
- Critically ill patients

Blood samples were obtained from 3 groups of patients in Yaoundé:

- Smear-negative patients in an area where malaria is endemic (Yaoundé),serving as a control group or Group M-

- Patients with malaria as determined by a positive malaria smear test (Group M+)
- Patients with malaria and on anti- malaria medication (quinine) (Group Mm).

Laboratory records of patients enrolled in the study were reviewed. A total of 105 blood samples were collected between March 2011 and June 2011. Nine samples were excluded because the patients were HIV positive, 6 were excluded due to haemolysis of the samples.
The investigation was performed on venous blood samples drawn into 5ml heparin coated tubes.

Laboratory blood testing:

A drop of each sample was used for a rapid HIV test and for preparation of the thick smear for malaria parasites.

Malaria diagnosis:

The diagnosis of malaria was performed by microscopic examination of blood films stained with Giemsa. The thick blood slides were examined by an experienced microscopist to determine presence or absence of *Plasmodium falciparum.*

Anti Malarial treatment:

A loading dose of Quinine dihydrochloride 20mg/kg by infusion over 4 hours in 5% dextrose was given followed by a maintenance dose: 8 hours after the start of the loading dose, 10 mg/kg quinine dihydrochloride infused over 4 to 6 hours and repeated every 8 hours until the patient could take oral quinine. After that, 2 tablets of quinine 300mg each were swallowed every 8 hours with food for 7 days.

Labelling Red Blood Cells:

Protocol:

The Brookhaven in vitro red blood cell labelling with Tc-99m method as described by Lackay [45] was used, with the following steps:

1. 2 ml of blood were withdrawn from each heparin coated tube and added to an in vitro red blood cell kit.
2. The RBC kit was incubated for 5 min on an automatic rotator.
3. After incubation, 0.6 ml of a 0.1% sodium hypochlorite solution was added to the mixture.
4. 1 ml Acid citrate dextrose solution A (ACD-A) was added to the vial.

5. Tc-99m pertechnetate (600 – 800 MBq) from generator eluate not older than 2 hours was added to the vial.
6. The kits were incubated for 15 min at room temperature and mixed carefully at approximately 5 min intervals.
7. Labelling efficiency (LE) was determined within 5 minutes after completion of incubation, after centrifugation of the vial for 5 min at 2000 revolutions per minute, removing the supernatant from the red blood cells and measuring the activity of red blood cells and supernatant in a dose calibrator.

Measurement:

The radioactivity of both the packed red blood cells and the supernatant were measured in a dose calibrator. Labelling efficiency was calculated as follows:

$$\%LE = \frac{ARbc}{ARbc + AS} x100$$

Analysis of data:

MS Excel was used to capture the data and non parametric data analysis was performed using Statistica software. Data was examined for significant departures from normality using a Shapiro-Wilks test. As the data are not distributed normally, the Kruskal-Wallis ANOVA test was used. The primary objective was to determine whether there is a significant difference between the labeling efficiency of the following groups:

- Control group
- Malaria group without anti-malarial medication
- Malaria group with anti-malarial medication (quinine).

CHAPTER FOUR

RESULTS

In this chapter, the results of the radiolabelling of blood samples includes those from individuals without malaria or control group (Group M-), those from patients with malaria (Group M+), as well as those from patients with malaria and on quinine therapy (Group Mm). A total of 90 individuals were enrolled in the study, both sex included. Thirty blood samples were without malaria, 30 others with malaria as determined by positive malaria smear test and 30 samples from patients with malaria and on quinine medication. All the blood samples were from individuals of the same geographic location. Each sample was labelled and their percentage labelling efficiency documented.

Full details of all patient blood samples including age, sex and percentage labelling efficiency are provided in table 1, table 2 and table 3 below.

Table 1 Blood sample details for the malaria free control group (M-)

Patient No	Sex	Age (years)	%RBC	Patient No	Sex	Age (years)	%RBC
1	M	45	93.81	16	M	24	99.32
2	F	22	98.49	17	M	39	98.67
3	M	23	97.97	18	M	23	99.16
4	F	30	94.93	19	F	53	99.19
5	F	30	99.40	20	F	23	98.94
6	F	35	97.82	21	M	28	97.89
7	M	22	94.58	22	M	36	99.81
8	F	18	88.71	23	F	34	97.72
9	M	26	98.69	24	F	40	99.50
10	F	18	98.63	25	F	24	99.81
11	M	57	99.22	26	M	25	98.99
12	M	42	99.37	27	F	42	99.48
13	F	51	99.46	28	M	51	99.72
14	F	18	99.70	29	M	41	98.52
15	F	18	99.65	30	F	42	98.70

Table 2 **Blood sample details for the malaria positive patient group (M+)**

Patient No	Sex	Age (years)	%RBC	Patient No	Sex	Age (years)	%RBC
1	M	25	99.48	16	F	20	98.93
2	F	54	99.84	17	M	22	99.20
3	M	27	85.80	18	M	28	99.15
4	F	20	96.99	19	F	25	99.07
5	F	22	99.64	20	F	30	98.32
6	M	25	99.70	21	M	25	98.21
7	F	20	99.85	22	F	55	99.48
8	M	28	98.99	23	F	30	99.62
9	M	28	94.85	24	M	19	99.01
10	M	25	99.62	25	M	41	98.52
11	M	27	99.15	26	M	42	99.48
12	F	30	99.50	27	F	51	99.72
13	M	24	99.32	28	F	32	99.53
14	F	38	98.41	29	F	28	99.11
15	M	31	99.88	30	F	30	99.00

Table 3 **Blood sample details for the malaria patients on quinine medication group (Mm)**

Patient No	Sex	Age (years)	%RBC	Patient No	Sex	Age (years)	%RBC
1	F	36	98.80	16	F	44	99.49
2	M	29	99.44	17	M	18	98.33
3	M	40	97.90	18	M	25	98.99
4	F	57	97.10	19	F	25	99.10
5	F	29	94.53	20	M	26	99.77
6	F	38	99.92	21	F	24	99.44
7	F	30	97.95	22	F	25	99.12
8	M	20	98.28	23	M	32	97.74
9	M	41	99.14	24	M	25	99.48
10	M	37	96.64	25	M	38	99.17
11	M	20	99.15	26	F	27	98.11
12	F	40	99.50	27	F	23	97.24
13	M	24	98.48	28	M	22	98.90
14	F	29	98.84	29	F	26	99.70
15	F	20	99.49	30	F	28	99.00

A summary of labelling efficiencies for patient samples with descriptive statistic analysis from each group as well as that from all three groups are provided in table 4 below.
The more complete tables with descriptive statistic analysis are provided in the addendum (table A_3, table A_4, table A_5 and table A_6).

Table 4: Descriptive statistics for the variable % labelling efficiency (%RBC) for the three groups

Group	Valid N	Mean	Std. Dev.
Control (M-)	30	98.19500	2.346495
M+	30	98.57900	2.610795
Mm	30	98.62467	1.135781
All groups combined	90	98.46622	2.114949

Statistical unweighted means analysis per group (see Addendum table A_1) showed that there was no significant difference in the mean ages of the 3 study groups. Individuals without malaria included in the study had a mean age of 32. 7 years. The mean age of the patients with malaria was 30.1 years and the group of patients having malaria and on quinine therapy had a mean age of 29.9 years.

The control group M- and the malaria therapy group Mm both had samples from 14 males and 16 females, while the group of patients with malaria but not on treatment (M+) consisted of 15 males and 15 females. Thus the sex of patients did not influence results (See analysis in table A_2 addendum).

Two dimensional histograms of labelling efficiencies for each group as well as for all groups together were plotted and are represented in figure 4, 5, 6 and 7 below. All the histograms present a unique sharp peak. The shape obtained has a non Gaussian distribution of data.

Figure 4: Histogram of percentage of labelling efficiencies in control group M-

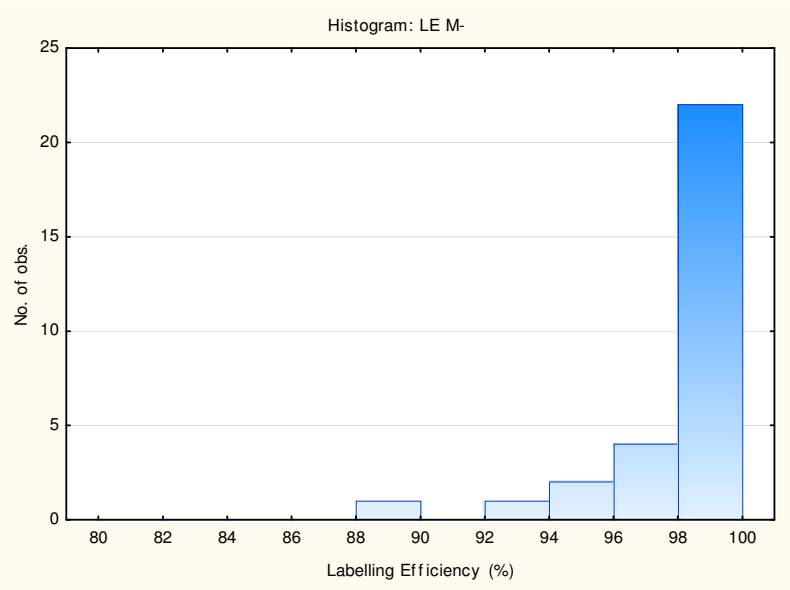


Figure 5: Histogram of percentage of labelling efficiencies in group M+

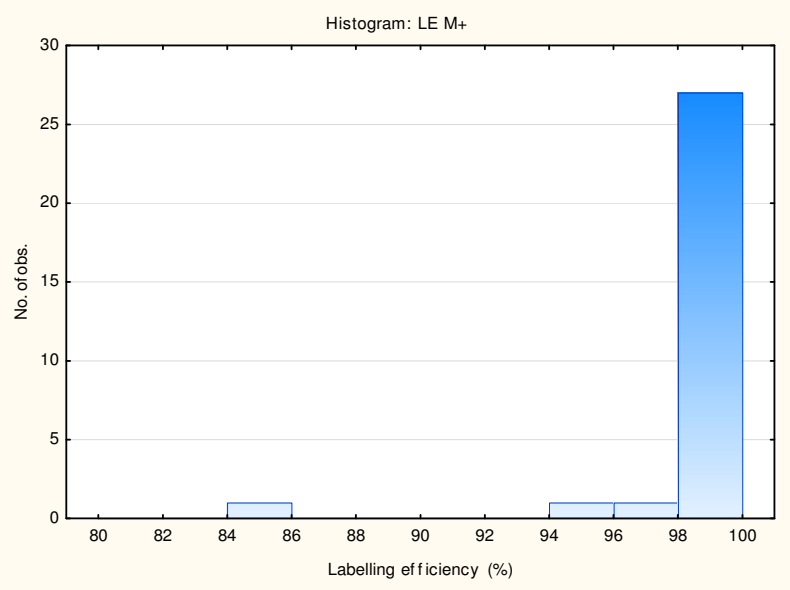


Figure 6: Histogram of percentage of labelling efficiencies in group (Mm)

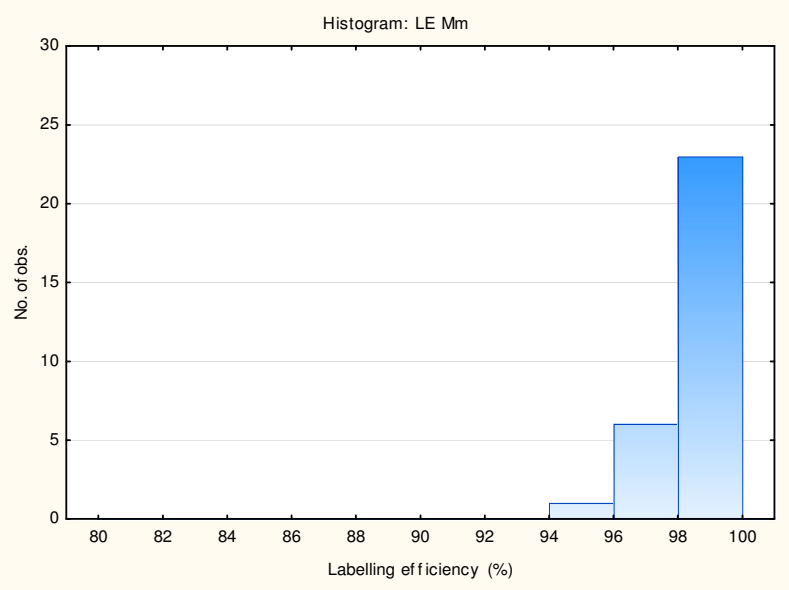
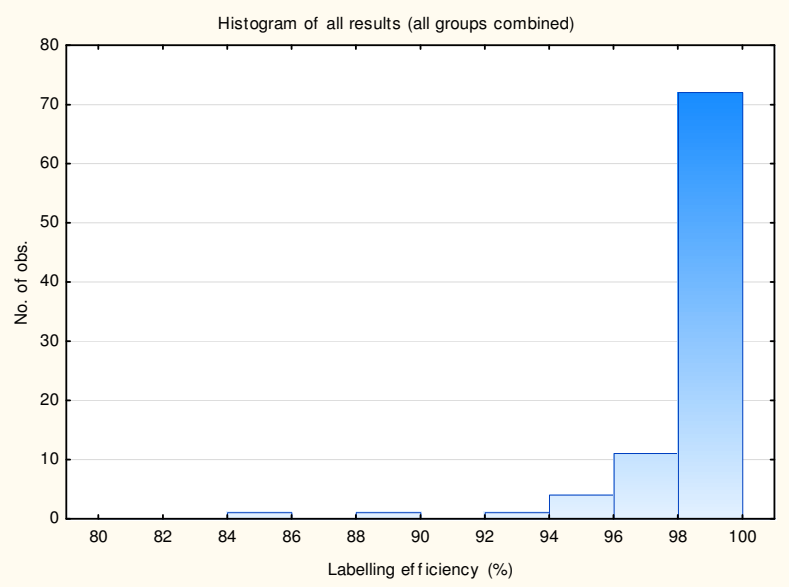


Figure 7: Histogram of percentage of labelling efficiencies in all groups



A non parametric data analysis using the Kruskal-Wallis ANOVA by Ranks test of the percentage of labelling efficiencies in all groups was performed. A P-value of 0.2117 was obtained as shown in table 5. This confirms that there is no significant difference between percentages of labelling efficiencies for the 3 groups.

Table 5: Non parametric data analysis:

Kruskal-Wallis ANOVA by Ranks; %RBC

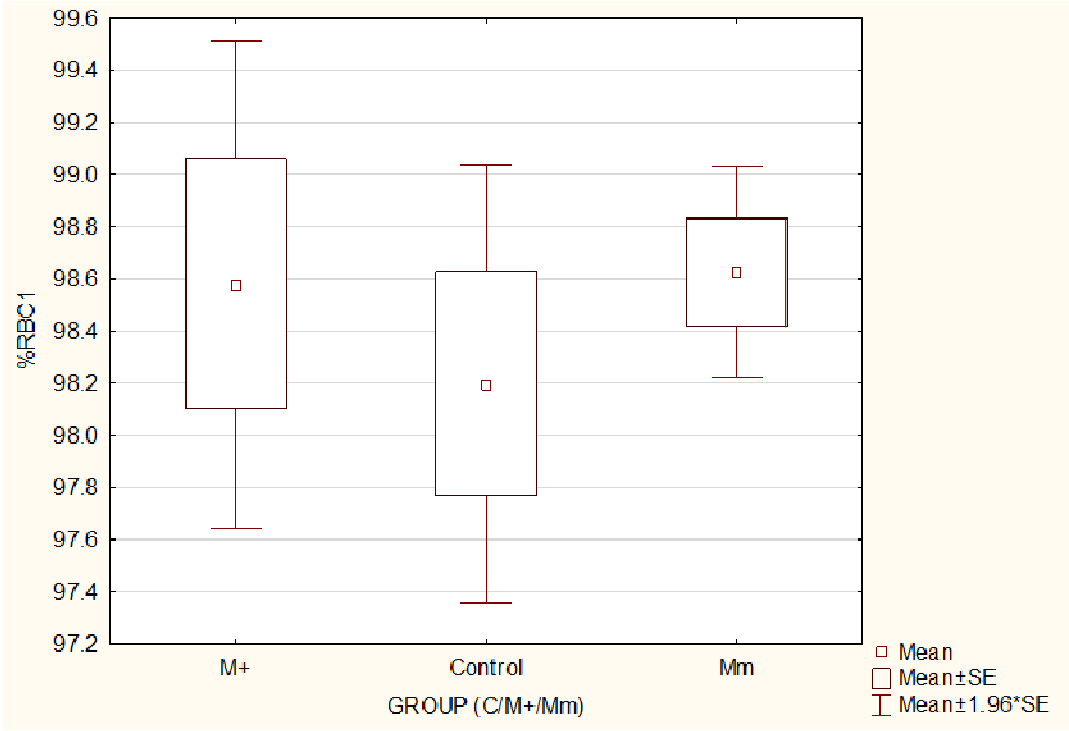
Independent (grouping) variable: GROUP (C/M+/Mm)

Kruskal-Wallis test: H (2, N= 90) =3.104885 p =0.2117

Depend.: %RBC	Code	Valid N	Sum of Ranks	Mean
Control	102	30	1278.500	42.61667
M+	101	30	1570.000	52.33333
Mm	103	30	1246.500	41.55000

The box plot in figure 8 illustrates the similarity of the results from the three experimental groups, with means that are very close to each other and overlapping standard errors.

Figure 8: Boxplot of Labelling efficiencies for the 3 groups



CHAPTER FIVE
DISCUSSION AND CONCLUSION

DISCUSSION

Nuclear Medicine studies using red blood cells labelled with Tc-99m are often used as it is an excellent tool for many diagnoses, including blood pool imaging and left ventricle ejection fraction investigations. Patients enrolled for such studies have concomitant diseases and are often on medication.

This study investigated the possible influence of malaria parasites and quinine on in vitro labelling of red blood cells. The mean labelling efficiency in our study was 98.2 ± 2.3% for the control group, 98.5 ± 2.6% in blood samples infested with malaria parasites, and 98.6 ± 1.1% in patients with malaria treated with quinine. Similar labelling efficiencies were documented by Spicer [7] with a mean LE of 97.8% for a control group and 98.8% for blood incubated with antineoplastic drugs. Both studies used the in vitro labelling technique.
The overall values of LE in all groups in the current study shows a great variability which was not sex or age related. The value of labelling efficiencies in the current study ranged between 85.8% and 99.9%. This broad range of values is not unexpected, and similar to the values documented by Lackay using the same labelling technique in a normal volunteer group, with values between 86.2% and 99.7% [45]. Although there is no prescribed or pharmacopoeial minimum value for red cell labelling efficiency, the producer of the UltraTag® kit states that 95% should usually be achieved [43], and 90% or more is usually regarded as acceptable for clinical studies [45]. In the present study as well as Lackay's study mentioned above, LE exceeded 95% for the majority of samples. In both control group and malaria positive group without medication, very few cases had unsatisfactory LE of lower than the typical value of 95%, with only one case for each group showing a very low LE between 85% and 90%.

The method of labelling and ingredients of the labelling kit, could affect the binding of Tc-99m in red blood cells. The in vitro method used in this study is documented as giving highest percentage of labelling, performing better than in vivo or in vivitro methods [29]. This is well shown in the current study with a high mean of labelling efficiencies of all groups combined (98.5%). According to Spicer, many of the drugs reported to interfere with other RBC labelling techniques, have not been found to interfere with the UltraTag® RBC method [7,

62]. It is probable that the in vitro method using sodium hypochlorite after pretinning of the cells provides high labelling efficiencies due to the large quantity of stannous ion that allows for maximum cell tinning, and the oxidation of extracellular tin by the presence of sodium hypochlorite.

A few diseases are reported to affect labelling of red blood cells with Tc-99m, including chronic lymphocytic leukaemia, non-Hodgkin's lymphoma, systemic lupus erythematous, and sickle cell anaemia as causes of suboptimal labelling efficiency [68]. However there is usually no explanation of the mechanism causing the low labelling efficiencies in these diseases. From the pathophysiological mechanism occurring in malaria, haemoglobin is the site of action of the parasite inside red blood cells [13, 14]. Haemoglobin is also the site where technetium is bound in the erythrocyte [69]. We therefore postulated that there could be interference when these two mechanisms occurred at the same time. The current study however could not prove a significant difference in labelling efficiencies when erythrocytes are labelled with Tc-99m in presence of *Plasmodium falciparum* (group M+ LE= 98.6%) or in absence of the disease (LE control group 98.2 %).

There are many reports of drugs interfering with red cell labelling, but the mechanism of interference is not often detailed. The possible explanations of effects of drugs on the labelling of blood elements with Tc-99m include a direct inhibition (chelating action) of stannous and pertechnetate ions, damage induced in the plasma membrane, competition of the ions for the same binding sites, possible generation of reactive oxygen species that could oxidize the stannous ion and/or direct oxidation of the stannous ion [70]. Lee et al in 1983 reported interference of RBC labelling with prazosin and digoxin, and thought that the reaction was associated with the fact that digoxin affects red cell membrane transport by inhibition of the Na^+/K^+-ATP-ase dependent pump [71]. According to Tatum et al [72] patients who had previously received iodinated contrast media produced a suboptimal red cell labelling efficiency. It was found that the mean labelling efficiency in those patients was 30% as opposed to 90% in patients who had not received the contrast media. It was then thought that the poor labelling efficiency could be due to an alteration in the redox potential of the stannous ion and therefore affect its role as reducer of technetium [40].

We postulated that quinine might affect red cell labelling with Tc-99m, as its site of action against *Plasmodium falciparum* is in the erythrocyte [13] where Tc-99m is bound to the

haemoglobin molecule. However, we could not demonstrate a significant effect, with the group Mm having a mean LE of 98.6% compared to 98.2% in the control group.

The literature available on drugs affecting LE varies from simple case reports, laboratory in vitro studies, and animal experiments, to experimental work in humans.
Many drug interferences are known from case reports, including a list summarised by Adalet [68]. The list includes drugs like penicillin, doxorubicin, cardiac drugs like prazosin, propranolol, hydralazine, methyldopa. Heparinized catheters and quinidine among others were also documented to give poor quality red cell labelling [68, 73]. Hambye and coworkers did an extensive review of large number of patients' gated blood pool images after in vivo RBC labelling. They found that intravenous heparin and chemotherapy significantly affected RBC labelling and image quality [74].
From case reports, it is usually not possible to gain much information on mechanisms of interference. The case reports are however very valuable as they can alert nuclear physicians to potential problems.

Many authors focus their work on laboratory in vitro studies mostly performed on animals or blood from animals [51, 54, 75]. Abreu and coworkers incubated rat blood samples in vitro with guava extract and found that the extract may have antioxidant action and alter the membrane structure involved in ion transport in the cells, thus decreasing the labelling of blood cells with Tc-99m [76]. Similar laboratory in vitro findings with rat blood incubated in presence of *Fucus vesiculosus* extract used in popular medicine suggested a decrease Tc-99m RBC labelling, due to products present in this natural product solution which may complex with stannous ions and pertechnetate and have direct or an indirect effect on their intracellular concentration or which may modify the plasma membrane and alter their transport into cells [77]. Braga and coworkers in 2000 incubated rat blood with extracts of *Thuta occidentalis*, *Peumus boldus* and *Nicotiana tabacum* to monitor their effect on Tc-99m labelled RBC. Using an in vitro technique, they found that some medicinal plants can affect the radiolabelling of RBC with Tc-99m [53]. Another animal experiment from Diré in 2006 suggested that *Sechium edule* extract is capable of maintaining the efficiency of labelling of blood elements with Tc-99m in diabetic status, although diabetes as such alters the radiolabelling of blood constituents by the generation of active metabolites with oxidant properties which may alter the function and the structures of proteins [78]. Such laboratory studies in animal models could be worthwhile to help to understand the drug interaction in

nuclear medicine procedures, avoiding obtaining poor quality images and therefore the necessity to repeat the examination increasing the radiation dose to the patient, or even misdiagnosis [77]. These studies do however have some limitations. In most cases blood samples were incubated in vitro with drugs or plant extracts [76, 53, 51, 54]. This means that the influence of metabolites was not studied. In addition, the relevance of the quantities of drugs or plant extracts incubated with blood to the clinical situation is not always clear.

Very limited literature is available describing experimental studies of drug effects on red cell labelling in humans as it is not acceptable to administer either medicines or radionuclides to human subjects purely for experimental studies. Yet human studies would provide the most realistic representation of what can occur in the Nuclear Medicine clinic. Obtaining blood samples from patients would to some extent overcome problems, but would also introduce the difficulty of controlling other factors such as dose regimen of the medication, the effects of concomitant medication, and possible effects of the actual diseases of the patients, to name but a few.

Pauwels et al observed poor RBC labelling on gated blood pool studies of patients who had received adriamycin (doxorubicin) a few hours before. They followed this up with in vitro labelling experiments, incubating blood samples with adriamycin, that showed varying labelling efficiencies, depending on the concentration of adriamycin and incubation time [79]. Gerson and coworkers studied the influence of anticoagulants used during blood sample collection for in vitro RBC labelling in a group of volunteers and found that EDTA adversely affected labelling efficiency. The same authors did an investigation on a modified Brookhaven method for in vitro red cell labelling, adding an initial centrifugation step, in a series of 50 patients including 12 who were treated with doxorubicin. This technique was not influenced by doxorubicin [80]. Spicer [7] investigated the ability of Ultratag RBC kit to label RBCs with pertechnetate in presence of various antineoplastic drugs including doxorubicin, 5-fluorouracil, cyclophosphamide, vincristine and cisplatin. The five drugs were used either alone or in combination and incubated at 37°C for 30 minutes with 2 ml samples of whole blood obtained from normal volunteers. After labelling with Tc-99m, the radiochemical purity of each sample was determined. The results showed a mean LE of 97.79% for the control group and LE of more than 98% for each drug individually with a value of 98.87% as labelling efficiency for all drugs combined. From their statistical analysis of the labelling efficiencies obtained, there were a slight difference in values from samples containing antineoplastic medications and the samples containing the doxorubicin metabolites compared to the control. However from a clinical point of view, this difference may not be relevant.

One limitation of Spicer's studies was the use of only a small number of samples. The largest part of information from Spicer's study [7] and Pauwel's work [79] was obtained from blood samples incubated with medication, not from patients being treated with the medicines. This would not represent the true situation of patients, although the concentration of medication added were carefully calculated to represent true concentrations in patients' blood. In order to study the effect of metabolites, Spicer also obtained blood samples from 3 patients 30 minute post injection of doxorubicin when the product should be approximately 70% metabolised. Thus only a very small study was done on a single drug in a fairly realistic clinical situation.

A more recent and accurate study performed by Lackay was carried out with 33 true patients on anti-tuberculosis medication with a follow up of the LE in the first phase of treatment (rifampicin, isonizid, pyrazinamide, ethambutol) as well as in the second phase of the treatment (only rifampicin and isoniazid) of the same patients. The patients' blood samples taken before the commencement of treatment served as their own controls. The investigation was extended to examine many other factors in order to elucidate the influence of possible confounding factors on the results. All the labellings were performed by the same operator, the time elapsed after each blood sample was taken until labelling started as well as the age of the sodium hypochlorite used during the labelling and the age of RBCs labelling kit was considered. This study showed no significant effect of anti-tuberculosis treatment on in vitro red cell labelling [45].

The current study of the effects of malaria parasites and quinine is similar to Lackay's work in the selection of actual patients with the disease treated with typical dosage regimens. Exactly such patients could present to Nuclear Medicine clinics in sub-Saharan Africa. The blood samples should show the effect of the intact drug and its metabolites.

All the labelling throughout the current study were performed by the same operator in order to avoid possible operator dependant variations like more complete removal of supernatant from one labelling to another. All blood samples were labelled within a three month period, using the same lots of RBC kits and reagents. Thus confounding factors which could influence LE were avoided as far as possible.

A limitation of the current study is that three different groups of people were used to represent the different clinical situations, i.e. without malaria, with malaria and with the disease and undergoing treatment. Paired testing of different influences on the same patient's blood could thus not be tested, as was done in Lackay's study of tuberculosis treatment [45]. Lastly it

could be argued that the fact that our control group was from a population in a malaria endemic area may have influenced results. A study with a control group made of people from a non-endemic population might strengthen the value of the current study.

CONCLUSION

The aim of this study was to determine whether or not the presence of *Plasmodium falciparum* in red blood cells and the action of the anti-malarial drug quinine on erythrocytes could affect labelling of red blood cells with technetium-99m. Our results show no significant difference in labelling efficiencies in patients without malaria, with malaria, and with malaria on quinine therapy.

The presence of malaria parasites or quinine in patients' blood should not affect Nuclear Medicine investigations when blood is labelled using an in vitro red blood cell labelling kit.

ADDENDUM

Table A_1: Table of patient age in each group
Current effect: F(2, 87)=.69719, p=.50074. Effective hypothesis decomposition

Group	Mean Age	Age Std.Err.	Age -95.00%	Age +95.00%	N
Control	32.66	1.845612	28.99831	36.33502	30
M+	30.06	1.845612	26.39831	33.73502	30
Mm	29.93	1.845612	26.26498	33.60169	30

Table A_2: Distribution of patients in sex in each group
Observed Frequencies

Sex	Group M+	Group M- (control)	Group Mm	Row Totals
M	15	14	14	43
F	15	16	16	47
Totals	30	30	30	90

Table A_3: Control group descriptive Statistics of %RBC with more details

Valid N	Mean	Confidence - 95.000%	Confidence 95.000%	Median	Std. Dev.
30	98.19500	97.31880	99.07120	98.96500	2.346495
Minimum	**Maximum**	**Lower Quartile**	**Upper Quartile**	**Percentile 10.00000**	**Percentile 90.00000**
88.71000	99.81000	97.97000	99.46000	94.75500	99.71000

Table A_4: Group M+ descriptive Statistics of %RBC with more details

Valid N	Mean	Confidence -95.000%	Confidence 95.000%	Median	Std. Dev.
30	98.57900	97.60411	99.55389	99.17500	2.610795
Minimum	**Maximum**	**Lower Quartile**	**Upper Quartile**	**Percentile 10.00000**	**Percentile 90.00000**
85.80000	99.88000	98.93000	99.62000	97.60000	99.78000

Table A_5: Group Mm descriptive Statistics of %RBC with more details

Valid N	Mean	Confidence - 95.000%	Confidence 95.000%	Median	Std. Dev.
30	98.62467	98.20056	99.04877	98.99500	1.135781
Minimum	**Maximum**	**Lower Quartile**	**Upper Quartile**	**Percentile 10.00000**	**Percentile 90.00000**
94.53000	99.92000	98.11000	99.44000	97.17000	99.60000

Table A_6: All groups descriptive Statistics of %RBC with more details

Valid N	Mean	Confidence - -95.000%	Confidence 95.000%	Median	Std. Dev.
90	98.46622	98.02325	98.90919	99.10500	2.114949
Minimum	**Maximum**	**Lower Quartile**	**Upper Quartile**	**Percentile 10.00000**	**Percentile 90.00000**
85.80000	99.92000	98.33000	99.48000	97.04500	99.71000

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